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Acaulospora reducta sp. nov. and *A. excavata* — two glomeromycotan fungi with pitted spores from Brazil

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Abstract — *Acaulospora reducta* sp. nov. and *A. excavata*, both characterized by large pits on their spore surfaces, were found in the semi-humid Atlantic rainforest and semi-arid Caatinga biomes of Northeastern Brazil. Phylogenetic analyses of the ITS regions of the ribosomal gene place the two fungi in two distinct clades within the *Acaulosporaceae*. *Acaulospora reducta* has whitish yellow, dark yellow to light brown spores (135–205 µm in diam.) and irregularly shaped, often edged to sometimes dumbbell-shaped pits (5.5–19 × 3.5–8.6 µm). These large pits have roughened irregular surfaces comprising secondary small pits (c. 0.5 µm broad and deep) and fine ridges. Spores of *A. excavata* are ochre to yellow orange (sometimes yellow) and have regular circular to subcircular pits (4–20 × 4–16 µm) with smooth pit surfaces. Most recently, *A. reducta* was also found in the Cerrado biome of Minas Gerais, suggesting its wide distribution in tropical Brazil.

Key words — *Glomeromycota*, *Diversisporales*, rDNA

Introduction

Recently, the number of new arbuscular mycorrhizal (AM) fungal species forming acaulosporoid (sensu lato) spores has greatly increased (e.g., Goto et al. 2008; Palenzuela et al. 2008, 2011; Błaszkowski 2012; Oehl et al. 2015), especially in the *Acaulosporaceae* due to concomitant morphological and molecular spore analyses. *Acaulospora* species have been increasingly reported both from colder climates (e.g. Oehl et al. 2011a, 2012; Palenzuela et al. 2013, 2015) and hot tropical areas (e.g., Goto et al. 2010, 2013; Furrazola et al. 2013; Oehl et al. 2014), with some species found in both cold and hot climates (e.g., Oehl et al. 2011b).

During surveys of AM fungal diversity in different agricultural and natural ecosystems in Brazil, two *Acaulospora* species were found with numerous conspicuously large depressions on the spore surfaces. *Acaulospora excavata*, which had been described from a similar climate zone in Ivory Coast (West Africa) and which was easily identified by large regular pits covering the spore surface (Ingleby et al. 1994), had never been subjected to molecular analyses. In *Acaulosporaceae* such prominent pits have rarely been found (e.g., for *A. foveata* Trappe & Janos and *A. bireticulata* F.M Rothwell & Trappe; Rothwell & Trappe 1979, Janos & Trappe 1982 as summarized in Błaszkowski 2012 and Oehl et al. 2012, 2014). The second fungus, new and described and illustrated herein as *Acaulospora reducta,* has similarly large pits that are more irregular and more complex than *A. foveata* and *A. excavata*. The objective of this study was to analyze the two fungi concomitantly on spore morphology and the ribosomal ITS region and to compare both with each other and other *Acaulospora* species.

Material & methods

Study area and sites

Soil samples were collected during the June 2011wet season and subsequent March 2012 dry season at the Itapirema Experimental Station, Agronomic Institute of Pernambuco – IPA, located in the municipality of Goiana (7°38′20″S 34°57′10″W; 13 m asl), Pernambuco State, NE Brazil. *Acaulospora reducta* was found in plantations of cassava (*Manihot esculenta* Crantz; *Euphorbiaceae*), sapodilla (*Manilkara zapota* (L.) P. Royen; *Sapotaceae*), and mahogany (*Swietenia macrophylla* King; *Meliaceae*) of the experimental station, while *A. excavata* was found only in the sapodilla and mahogany plantations. The climate in this sampling area is Ams' (after Köppen; Kottek et al. 2006), semi-humid with a tropical monsoon and a dry summer. Mean annual precipitation is around 2000 mm with a mean annual temperature of 24°C. The soil type is a Ferralsol (IUSS Working Group WRB, 2014). Pereira et al. (2014) describe the soil chemical characteristics; the mean soil pH (H_2O) was 6.1, organic carbon 7.7 g kg^{-1}, and available P 11.0 mg kg^{-1}.

Acaulospora reducta was additionally found in a coastal 'restinga' vegetation in Tamandaré (Pernambuco State; 8°46′52″S 35°06′22″W) and Mataraca (Paraíba State; 6°29′S 34°56′W), in a semiarid area with Caatinga vegetation in Petrolina (Pernambuco State; 9°03′16.68″S 40°18′59.23″W; 389 m asl) and Assú (Rio Grande do Norte State; 5°34′38″S 36°54′30″W; 60 m asl), and in Cerrado Ferralsol soil samples from Sete Lagoas (Minas Gerais State; 19°27′46″S 44°10′24″W; 738 m asl). The Cerrado soil was collected in May 2012 at the Embrapa Maize and Sorghum Experimental Station from a subsoil layer and used to prepare substrate for "On Farm" AM fungal inoculum production using maize (*Zea mays* L.) and *Urochloa decumbens* (Stapf) R.D. Webster as host plants. Mean soil pH was 5.4 (H_2O) with organic carbon 0.29 g kg^{-1} and available P 0.85 mg kg^{-1}. Lastly, the fungus was also found in Planaltina (near Brasilia) in a no-till production site grown with soybean (Distrito Federal, 15°36′S 47°42′W; 1014 m asl) and in a Sorghum production site near the type location in Itapirema (Pernambuco).

Acaulospora excavata was additionally found in a small home garden in Recife (Pernambuco State; 7°53′06″S and 34°50′11″W) within the rhizosphere of "Palmeira" (*Dypsis lutescens* (H. Wendl.) Beentje & J. Dransf.; *Arecaceae*), a palm known variously as bamboo palm, golden cane palm, areca palm, yellow palm, or butterfly palm.

Arbuscular mycorrhizal cultures

The native AM fungal communities from the sampling sites in Itapirema were propagated in trap cultures over three consecutive 4-month cycles using millet (*Panicum miliaceum* L.), maize, and sunflower (*Helianthus annuus* L.) as hosts in 2 L plastic pots according to Pereira et al. (2014). At the end of each cycle, the plants were allowed to dry and cut off, with 50 g soil aliquots collected for spore extraction. The soil was reseeded for the subsequent cycle. The cultures were maintained at the greenhouse of the Mycology Department, at the Federal University of Pernambuco, Recife, Brazil. However, the two fungi under study did not sporulate within three consecutive propagation cycles.

Morphological analyses

Spores of the two fungi were extracted from the field soil samples by wet sieving and sucrose centrifugation according to Sieverding (1991), separated under a stereomicroscope, and mounted in polyvinyl alcohol–lacto–glycerin (PVLG) and PVLG + Melzer's reagent (Brundrett et al. 1994). Slide mountants were dried (3–5 days at 60°C) to clarify oil drops and observed with a Nikon compound microscope (Eclipse Ni). The spore terminology follows Oehl et al. (2012), Furrazola et al. (2013), and Goto et al. (2013) for acaulosporoid glomeromycotan fungi.

Transmission electron microscopy

The samples of the new fungus were dehydrated by immersion in solutions of increasing concentrations of ethanol (25%, 50%, and 90%) for thirty minutes each followed by three baths with 100% ethanol. The samples were then immersed in three 30-minute baths in 100% acetone. Dehydrated spores critically point dried in an Emitech K850, sputter coated with gold in an Emitech K550, and examined under a Zeiss DSM 940 A scanning electron microscope at an accelerating voltage of 5–15 kV. The analyses were carried out at the Nucleus of Applied Biology microscopy lab at Embrapa Maize and Sorghum.

Molecular analyses

After isolation from field soil samples, spores from the two fungi were washed in ultrapure water and sonicated three to four times. The DNA was extracted from individual spores placed on a slide in a drop (5–10 µl) of ultrapure water, and crushed with a sterile needle. Crude DNA extract was used as template for a semi-nested PCR using the primers ITS5+LSUAC (White et al. 1990; 5′-CCATTACGTCAGCATCCTTAGCG-3′) and ITS1+28G6 (White et al. 1990; 5′-CGGGATTCTCACCCTCTATG-3′) consecutively.

PCR reactions were carried out in a volume of 50 µl, containing 75 mM Tris-HCl pH 8.8, 200 mM $(NH_4)_2SO4$, 0.01% Tween 20, 2 mM $MgCl_2$, 200 µM each dNTPs, 1 µM of each primer, and 2 units of DreamTaq DNA polymerase (Thermo Scientific, Maryland, USA); cycling parameters were one cycle of 5 min at 95°C; 40 cycles of 45 s at 94°C, 1 min at 55°C, and 1 min at 72°C; and a final elongation of 7 min at 72°C following the last cycle. The amplified products (~600) were purified with a GeneJET PCR Purification Kit (Thermo Scientific, USA) and cloned with a CloneJET PCR Cloning kit (Thermo Scientific, USA) following the manufacturer's instruction, and then sequenced by the Central Laboratory of the CCB/UFPE (Recife, Brazil). The phylogeny was inferred by ITS sequence analyses. These AM fungal sequences were then aligned with other glomeromycotan sequences from GenBank in ClustalX (Larkin et al. 2007). Sequences from *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüssler were used as outgroup. Prior to phylogenetic analysis, the model of nucleotide substitution was estimated using Topali 2.5 (Milne et al. 2004). Bayesian (two runs over 3×10^6 generations with a burn in value of 25%) and maximum likelihood (1000 bootstrap) analyses were performed, respectively, in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003) launched from Topali 2.5, using the GTR + I model. Maximum parsimony analysis was performed using PAUP*4b10 (Swofford 2003) with 1000 bootstrap replications.

Taxonomy

Acaulospora reducta Oehl, B.T. Goto & C.M.R. Pereira, **sp. nov.** FIGS 1–11

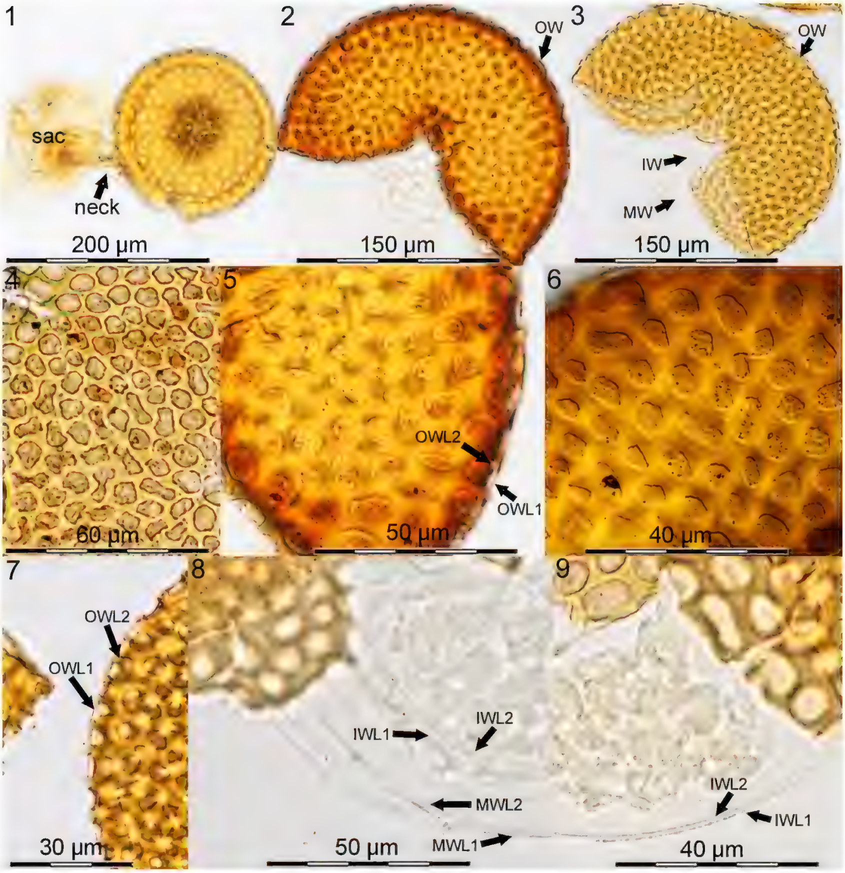
MYCOBANK MB 812332

> Differs from *Acaulospora excavata* by its more irregular pits on the outer spore wall that have roughened and slightly pitted inner surfaces.
>
> TYPE: Brazil, Pernambuco State, Goiana, Experimental Station of Itapirema (Instituto Agronômico de Pernambuco), from a Ferralsol in a cassava plantation, 1.6.2011, coll. C.M.R. Pereira (**Holotype**, URM87697; **isotypes**, URM87698, ZT Myc 55894; GenBank KM057063–KM057067).
>
> ETYMOLOGY: *reducta* (Latin), referring to the small depressions within the large pits on the outer spore wall.

SPOROCARPS unknown. Acaulosporoid spores form singly in soils laterally on the tapering hyphal neck of sporiferous saccules.

SPORIFEROUS SACCULES are hyaline to subhyaline to light yellow, rarely dark yellow, globose to subglobose, 140–210 µm in diam. and form terminally on hyphae. They are generally detached from mature spores. The saccule wall generally is bi-layered with a rapidly degrading evanescent hyaline to light yellow outer layer (0.5–1.5 µm) and a semi-persistent sub-hyaline to light yellow to dark yellow inner layer, which is 1.8–2.8(–4.4) µm.

SPORES form singly on the saccule necks. They are globose (135–205 µm diam.) to subglobose to oval (145–210 × 130–195 µm), whitish yellow to dark yellow. The spores form three walls: outer wall (OW), middle wall (MW), and inner wall (IW).



Figs 1–9. *Acaulospora reducta*. 1. Spore formed laterally on the neck of a sporiferous saccule (sac); globose saccule terminus already collapsed; 2–3. Crushed spores with three walls: pitted outer wall (OW), smooth middle wall (MW) and granular inner wall (IW); 4–6. Large, irregular pits on the outer OW surface in planar view showing a roughened, irregular inner surface structure resembling small depressions and projections of c. 0.5–1.5 µm in diam. and depth; 7. Pits on OW in cross view; OW with two layers visible (OWL1–2); inner, small pits not clearly visible within the large depressions; 8–9. MW and IW with multiple layers visible (MWL1–2; IWL1–2).

Outer wall consists of three layers, with the outermost layer (OWL1) evanescent to semi-persistent, 1.5–2.6 µm thick, hyaline to subhyaline. In older spores, the outer wall layer (OWL1) might degrade completely, but usually some fragments remain on the OWL2 surface. OWL2 is laminated, 6.0–14.5 µm thick, whitish yellow, dark yellow to light brown and has innumerous irregularly-shaped, often edged to sometimes dumbbell-shaped pits (5.5–19 ×

3.5–8.6 µm) that itself have roughened surfaces consisting of small depressions of approx. 0.5–1.5 µm in diam. and 0.5–1.5 µm depth) surrounded by projections of similar dimensions to fine ridges. OWL3 is thin (0.7–1.2 µm), yellowish and tightly attached to OWL2. None of the OW layers stains in Melzer's reagent.

MIDDLE WALL is bi-layered, hyaline, semi-flexible to flexible and in total 2.0–3.5 µm thick. MWL1 is 1.0–1.7 µm thick, and generally tightly adherent to MWL2, which is 1.0–1.8 µm. None of the MW layers stains in Melzer's reagent.

INNER WALL is three-layered, hyaline, and in total 3.6–5.8(–9.2) µm thick. The outer layer IWL1 is 0.6–1.3 µm, with a granular ('beaded') structure, and tightly adherent to IWL2, which is 2.5–3.5 µm thick and sometimes, as being 'amorphous', expands up to 6.9 µm in lactic acid based mountants. IWL3 tightly adheres to IWL2, and is, as only 0.5–1.0 µm thick, usually difficult to detect. IWL2 stains dark red purple in Melzer's reagent, but this stain may disappear completely within months in the mountants.

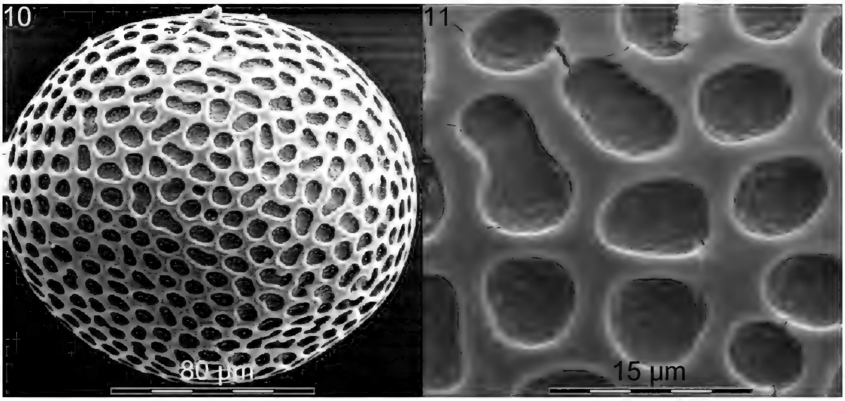
GERMINATION was not observed.

ARBUSCULAR MYCORRHIZA FORMATION so far unknown.

ADDITIONAL SPECIMENS EXAMINED: **BRAZIL**, **PERNAMBUCO STATE**, Goiana, Experimental Station of Itapirema, from a sapodilla plantation, 1.6.2011, coll. C.M.R. Pereira (URM87699); from a mahogany plantation, 1.6.2011, coll. C.M.R. Pereira (URM87700); from a *Sorghum bicolor* production site, coll. D.M.A. Assis, 18.8.2015 (URM88218); Tamandaré, from a 'restinga' vegetation (1.6.2013, coll. D.K.A. Silva (URM88213); Petrolina, from a natural Caatinga, 1.6.2014, coll. D.K.A. Silva (URM88214); **PARAÍBA STATE**, Mataraca, from a 'restinga'; 1.6.2012, coll. D.K.A. Silva (URM88215); **RIO GRANDE DO NORTE STATE**, Assú, from a natural Caatinga, 15.9.2013, coll. A.S. Medeiros (URM88216). **MINAS GERAIS STATE**, Sete Lagoas, from a natural Cerrado forest savanna, 1.5.2012, coll. F.A. Souza (URM88217, ZT Myc 55273); **DISTRITO FEDERAL**, Planaltina, from a no-till production site with soybean, 1.3.2014, coll. J.S. Pontes (URM88219).

PHYLOGENETIC ANALYSES — rDNA sequence analysis of the ITS region inferred the phylogeny of *Acaulosporaceae,* placing *A. reducta* in a monophyletic clade with high levels of support near *A. scrobiculata* and *A. minuta* and supporting *A. reducta* as a new species within *Acaulospora* (FIG. 12). In BLASTn analysis, the species most closely related to *A. reducta* was *A. scrobiculata* (FR692353) with 97% identity; no environmental ITS sequences deposited in GenBank corresponded to *A. reducta.*

DISTRIBUTION — *Acaulospora reducta* has been recorded from the semi-humid Atlantic rainforest, the semi-arid Caatinga, and the Cerrado savanna biomes of tropical Brazil. In the Atlantic Rainforest biome, the fungus was isolated from agricultural systems in Itapirema (Goiana, Pernambuco State) and coastal sand dunes with typical 'restinga' vegetation in Tamandaré (Pernambuco State) and Mataraca (Paraíba State). In the Caatinga biome, it was found in regeneration areas from the National Forest Flona de Açu in Assú



FIGS 10–11. *Acaulospora reducta* (transmission electron microscopy). 10. Uncrushed spore with innumerous large pits on the spore surface and multiple small depressions and projections on the pit surfaces; 11. Spore segment showing detail view of the multiple small depressions and projections within the large pits.

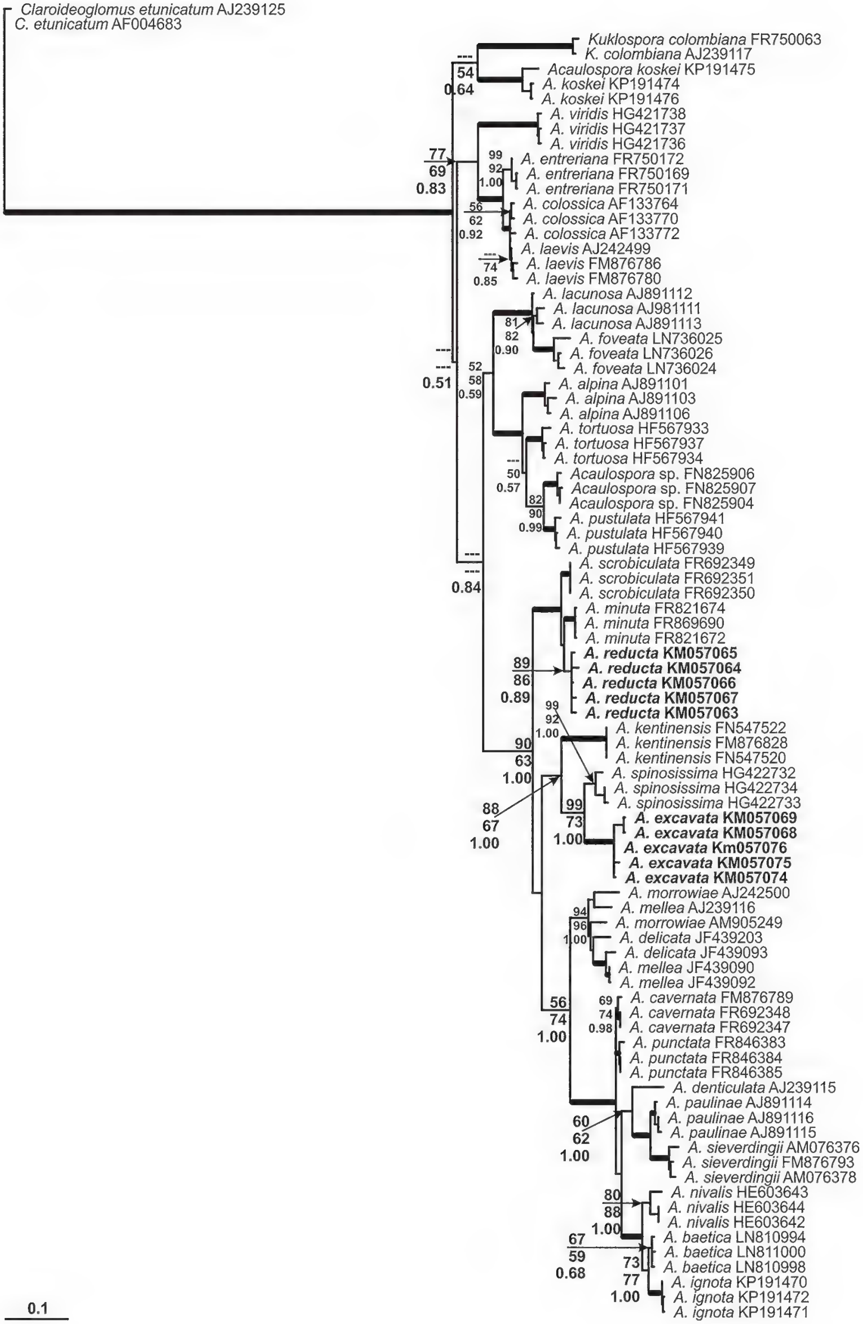
(Rio Grande do Norte State) and in a preserved Caatinga area in Petrolina (Pernambuco State). Finally, in the Cerrado biome it was found in a forest savanna of Sete Lagoas (Minas Gerais State). It was also found in mycorrhizal inoculum production cultures of non-sterile soils, derived from this Cerrado area and cultivated with maize and *Urochloa decumbens*. The fungus co-occurred at the type location with *A. excavata*, among many other AMF species (see Pereira et al. 2014).

Acaulospora excavata Ingleby & C. Walker, Mycotaxon 50: 100. 1994 FIGS 13–16

MYCOBANK MB 361679

Spores form laterally on the necks of sporiferous saccules, which are 110–190 × 100–165 µm. Spores are globose to subglobose to oval (115–195 × 100–180 µm), pale ochraceous to pale yellow to yellow orange to sometimes bright yellow. The spores form three walls: triple-layered outer wall (OWL1-3), bi-layered middle wall (MWL1-2), and triple-layered inner wall (IWL1-3). OWL2 with circular to elliptical pits (3.9–20 × 3.5–16 µm in diam.; 2.2–6.3 µm in depth). IWL1 with 'beaded', granular structure; IWL2 staining purple to dark purple in Melzer's reagent.

SPECIMENS EXAMINED — **COTE D'IVOIRE**, southwest of Tissale, Mopri Forest Reserve, under *Terminalia ivorensis*, 30 Oct 1990, C. Walker 1674 (OSC 83345, isotype). **BRAZIL, PERNAMBUCO STATE**, Goiana, Experimental Station of Itapirema (Instituto Agronômico de Pernambuco), from a Ferralsol in a sapodilla plantation, 1.6.2011, coll.: C.M.R. Pereira (URM87701), and from a mahogany plantation, 1.6.2011, coll.: C.M.R. Pereira (ZT Myc 55895); **PERNAMBUCO STATE**, Recife, in the rhizosphere of


Claroideoglomus etunicatum AJ239125
C. etunicatum AF004683
Kuklospora colombiana FR750063
K. colombiana AJ239117

54
0.64
Acaulospora koskei KP191475
A. koskei KP191474
A. koskei KP191476
A. viridis HG421738
A. viridis HG421737
A. viridis HG421736
77
69
0.83
99
92
1.00
A. entreriana FR750172
A. entreriana FR750169
A. entreriana FR750171
56
62
0.92
A. colossica AF133764
A. colossica AF133770
A. colossica AF133772
A. laevis AJ242499

74
0.85
A. laevis FM876786
A. laevis FM876780
A. lacunosa AJ891112
A. lacunosa AJ981111
81
82
0.90
A. lacunosa AJ891113
A. foveata LN736025
A. foveata LN736026
A. foveata LN736024

0.51
52
58
0.59
A. alpina AJ891101
A. alpina AJ891103
A. alpina AJ891106
A. tortuosa HF567933
A. tortuosa HF567937
A. tortuosa HF567934

50
0.57
Acaulospora sp. FN825906
Acaulospora sp. FN825907
Acaulospora sp. FN825904
82
90
0.99
A. pustulata HF567941
A. pustulata HF567940
A. pustulata HF567939

0.84
A. scrobiculata FR692349
A. scrobiculata FR692351
A. scrobiculata FR692350
A. minuta FR821674
A. minuta FR869690
A. minuta FR821672
89
86
0.89
A. reducta KM057065
A. reducta KM057064
A. reducta KM057066
A. reducta KM057067
A. reducta KM057063
99
92
1.00
A. kentinensis FN547522
A. kentinensis FM876828
A. kentinensis FN547520
90
63
1.00
88
67
1.00
A. spinosissima HG422732
A. spinosissima HG422734
A. spinosissima HG422733
99
73
1.00
A. excavata KM057069
A. excavata KM057068
A. excavata Km057076
A. excavata KM057075
A. excavata KM057074
A. morrowiae AJ242500
A. mellea AJ239116
94
96
1.00
A. morrowiae AM905249
A. delicata JF439203
A. delicata JF439093
A. mellea JF439090
A. mellea JF439092
56
74
1.00
69
74
0.98
A. cavernata FM876789
A. cavernata FR692348
A. cavernata FR692347
A. punctata FR846383
A. punctata FR846384
A. punctata FR846385
A. denticulata AJ239115
60
62
1.00
A. paulinae AJ891114
A. paulinae AJ891116
A. paulinae AJ891115
A. sieverdingii AM076376
A. sieverdingii FM876793
A. sieverdingii AM076378
80
88
1.00
A. nivalis HE603643
A. nivalis HE603644
A. nivalis HE603642
67
59
0.68
A. baetica LN810994
A. baetica LN811000
73
77
1.00
A. baetica LN810998
A. ignota KP191470
A. ignota KP191472
A. ignota KP191471
0.1

Dypsis lutescens, 1.7.2012, coll.: I.P. de Monte Júnior (URM87702, GenBank KM057068–KM057069; KM057074–KM057076).

Notes — Our morphological analyses revealed no differences between the type specimens of *A. excavata* isolated from Mopri Forest Reserve in Ivory coast (West Africa) and our specimens isolated from Goiana and Recife, in Northeastern Brazil (Figs 13–16). Our study is the first to present molecular phylogenetic data for *A. excavata* (Fig. 12).

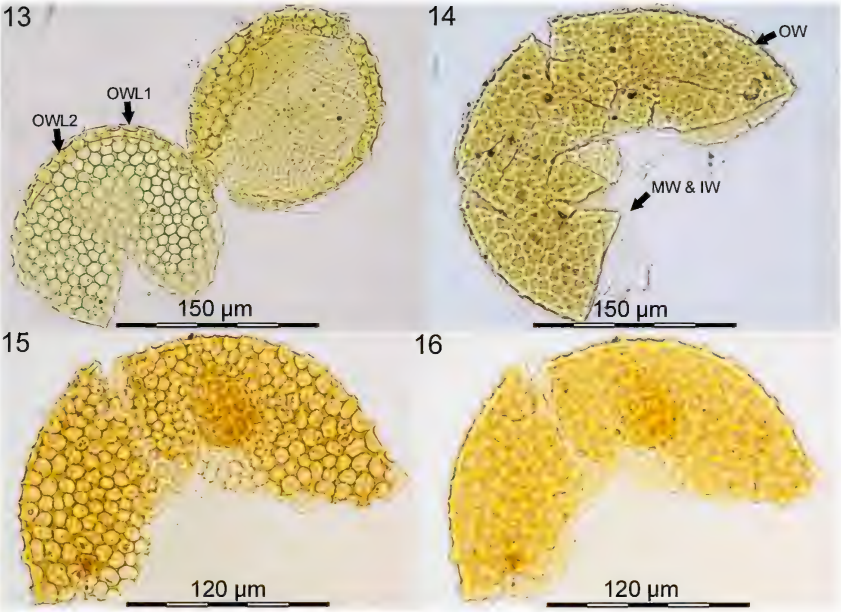
Phylogenetic analyses — The ITS sequence analysis grouped *A. excavata* with *A. spinosissima* and *A. kentinensis*. In BLASTn analysis, the sequences closest to *A. excavata* were from *A. spinosissima* (HG422733–HG422734) with 94% identity. No environmental ITS sequences deposited in GenBank corresponded with *A. excavata*.

Discussion

Acaulospora reducta and *A. excavata* represent two species with characteristic large pits on the outer spore wall that easily distinguish them from all other species in the *Acaulosporaceae*. While *A. excavata* has somewhat regularly sized pits (concerning both diameter and depth) and smooth inner pit surfaces, *A. reducta* has more irregular and often edged pits with roughened surfaces consisting of small depressions and projections inside the large pits, so that the pits are doubly ornamented, a diagnostic character within the *Glomeromycota*.

There are only a few other *Acaulospora* species that have pits close to as large as those found in *A. reducta* and *A. excavata*, such as *A. bireticulata*, *A. foveata*, and *A. verna* (Rothwell & Trappe 1979, Janos & Trappe 1982, Błaszkowski 2012). *Acaulospora foveata* has pitted spores similar to *A. excavata*, but its spores are reddish brown to brownish black and generally larger than those of *A. reducta* and *A. excavata* (Janos & Trappe 1982, Ingleby et al. 1994), and the pits are generally more irregular in *A. foveata* than in *A. excavata* (Trejo et al. 2015). *Acaulospora bireticulata* has light brown to brown spores with a double reticulum on the outer OW surface and regular and conspicuous spines within the large pits (Rothwell & Trappe 1979, Walker & Trappe 1981). *Acaulospora verna* also has rather large pits (3.2–7.8 µm if circular to subcircular, or 3.2–4.2 × 9.8–16.2 µm if ellipsoidal to oblong), but its spores are clearly smaller (60–95 µm) than those of *A. reducta* and the pit surfaces are smooth (Błaszkowski 2012).

Fig. 12. Phylogenetic tree of the *Acaulosporaceae* obtained by analyses from ITS1, 5.8 rDNA, and ITS2 sequences of different *Acaulospora* spp. Sequences are labeled with their database accession numbers. Support values (from top) are from maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses. Sequences obtained in this study are in boldface. Only support values of at least 50% are shown. Thick branches represent clades with more than 95% of support in all analyses. The tree was rooted by *Claroideoglomus etunicatum*. (Consistency Index = 0.50; Retention Index = 0.86).



Figs 13–16. *Acaulospora excavata.* Crushed spores with three walls: pitted outer wall (OW), smooth middle wall (MW) and granular inner wall (IW); large pits on the outer OW surface, with smooth surfaces within the pits. 13–14. OSC 83345, Côte d'Ivoire; 15. URM87701, Brazil; 16. URM87702, Brazil.

Other species (e.g., *A. cavernata,* Błaszkowski 1989; *A. elegans,* Gerdemann & Trappe 1974) have significantly smaller pits than *A. reducta* and *A. excavata.*

Phylogenetically, *A. reducta* and *A. excavata* form two distinct clades within *Acaulosporaceae*. Interestingly, they do not group closely together (Fig. 12) nor with *A. foveata,* which was recently analyzed from its type location in Veracruz, Mexico (Trejo et al. 2015). It would nonetheless be worthwhile to analyze also *A. excavata* from its type location in Ivory Coast, West Africa (Ingleby et al. 1994), from a climatic zone comparable to semi-humid tropical South America. Its distinctive morphology (Figs. 13–16), however, does support our species identification of the *A. excavata* isolates from Pernambuco (Brazil).

Acaulospora reducta is known from three biomes in NE and SE Brazil, the semi-humid Atlantic rainforest, the semi-arid Caatinga and the Cerrado savanna biomes. These findings suggest that *A. reducta* is widely distributed in tropical Brazil. The morphology of *A. excavata*, originally described from tropical West Africa, supports its occurrence in tropical South America. In the present study, molecular phylogenetic data of this fungus were shown for the first time.

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